Dermal exposure and uptake of chemicals for systemic risk assessment
A white paper
(prior to OEESC-2005 workshop)
(March 2005)

The purpose of this white paper is to provide context for the discussions in Workshop 2 "Quantitative Risk Assessment" of the Occupational and Environmental Exposure of Skin to Chemicals - 2005 conference (Stockholm, 12-15 June 2005). Recent activities are described. Then, a brief description is presented of current knowledge and knowledge gaps in the areas of dermal exposure and dermal absorption. Finally, the plans for the Workshop discussion are outlined.

Some recent activities

Recent studies, specifically the RISKOFDERM project, have established a large database of dermal exposure levels of chemicals in several occupational use scenarios (Ann. Occup. Hyg., 48/3 (2004)). The methods used to obtain these measurements were based on the OECD Guidance Document OCDE/GD (97)/148. Data were obtained over (part of) a work shift using state-of-the-art methodology that assessed the potential dermal exposure. This approach provides estimates of the mass of contaminant chemical that may be available to be taken up into the body, but does not take account of the protective effect of clothing or the mass of chemical that is likely to be taken up into the body. Estimation of uptake of chemicals through the skin is most relevant for risk assessment where an evaluation of systemic exposures is essential.

Other recently completed research, for example the EDETOX project (http://www.ncl.ac.uk/edetox/index.html), has focused on evaluation of permeation of chemicals through the skin. These studies have used both *in vitro* and *in vivo* assessment methodologies to assess the dermal uptake of chemicals. However, it is impractical to measure dermal permeation for the many thousands of industrial chemicals in use today. An alternative approach that has been proposed is to use predictions of steady-state permeation from statistically derived relationships between physical-chemical properties and the permeability coefficient of representative chemicals, relationships known as QSARs (quantitative structure-activity relationships).

In 2004 the European Chemical Industry Council (CEFIC) sponsored a scientific workshop to discuss skin permeation measurement methods (http://www.iom-world.org/news/ppworkshop.php). The workshop discussed, amongst others, the use of QSAR for risk assessment purposes. The data currently used for QSARs are obtained from "infinite dose" *in vitro* absorption studies, i.e. there is no limit to the amount of permeating chemical. Such studies determine the maximum flux (for the applied concentration), and from that flux a permeability coefficient k_p is calculated. The permeability coefficients for a set of chemicals are related by QSARs to physical-chemical properties such as octanol-water partition coefficient and molecular weight. However, realistic risk assessment scenarios usually correspond to "finite dose" conditions, plus current exposure assessment methods do not measure the concentration of contaminant chemicals.

The main immediate need identified by the CEFIC workshop was to establish the link between finite and infinite dose experiments, thus linking the QSAR derived information with the inputs required for risk assessment. The linkage between finite and infinite dose experiments relies on mathematical modelling and the associated relevant and reliable experimental data. These techniques enable a sound theoretical basis to be used in the interpretation of the data, and this should improve the reliability of parameters calculated from experimental data. The models also should enable extrapolation to predict absorption under different dosing conditions.

Up till now, there has been surprisingly little interaction between the researchers involved with occupational dermal exposure assessment and those researchers working on dermal permeation. This may be an important reason why there is a mismatch between the external exposure data obtained in the field or through modelling attempts, even after correction for clothing penetration, and experimental or QSAR data on dermal permeation.

Dermal exposure

Humans are dermally exposed to environmental contaminants via three media of exposure water, soil, and air and as pure chemicals or mixtures in occupational settings. The site of dermal exposure is directly related to the activity being performed at the time of exposure. Several factors can influence dermal exposure during activities. These include:

- reduction or increases in the chemical contact with skin due to normal clothing;
- protective clothing and gloves worn by workers and the amount of protection they offer;
- individual differences in dermal exposure due to differing degrees of speed, care, and dexterity in performing work;
- variance in the amount of material available for dermal absorption due to actions such as wiping the affected area with the hand;
- variances in the penetrability of the skin in different parts of the body;
- individual variability in regards to skin penetrability due to age and skin condition, such as disease and thickness of the stratum corneum; and
- the matrix of the chemical contaminant, solid, liquid, or vapour.

Dermal exposure is defined as the process of contact between an agent and skin at an exposure surface over an exposure period. The (target) exposure surface in view of the dermal route is the skin contaminant layer (SCL) compartment, i.e. the compartment on top of the stratum corneum of the human skin, and is formed by sebum lipids, sweat and additional water from transepidermal water loss, rest products from cornification and unshed corneocytes, and is given by its three dimensional volume.

Parameters of the result of contact are: dermal exposure mass, i.e. the mass of agent present in the contact volume; dermal exposure loading, i.e. exposure mass divided by the skin surface area where an agent is present; dermal exposure concentration, i.e. exposure mass divided by the exposure volume (SCL) or the exposure mass divided by the mass contained in the SCL.

The current dermal exposure assessment methodology should be improved so that biologically relevant data are to be collected. The current methodology is mainly based on assessing total exposure mass. Measurement methods for dermal exposure assessment, i.e. to identify and quantify an agent, can be grouped according to three major principles:

- sampling by interception of agent mass transport towards clothing and/or skin by the use of collection media (pads) placed at the skin surface or replacing (work) clothing during the sampling time followed by Detection, e.g., chemical analysis of extracts from the collection matrix;
- sampling by removal of the agent mass from the skin surface (SCL) at any given time or the end of the sampling period (by wash liquid, wipe fabrics, etc.), followed by detection in the collection matrix;
- direct assessment by *in situ* detection of the agent or a tracer at the skin surface, e.g. by image acquisition and processing systems, at a given time.

Since *in situ* techniques also determine the surface areas actually exposed, the results also indicate exposure loading of the SCL, whereas the results of removal techniques can be used to estimate exposure loading of the skin surface.

Mass transport processes can be divided into processes towards the clothing and skin compartments and processes from clothing and skin compartments. The latter are subdivided into two pathways: from the skin contaminant layer into the skin by uptake, and transport from the skin contaminant layer to other compartments by removal, resuspension or evaporation. High or low transport rates will bias the results obtained by different sampling methods. Low transport rates allow use of removal and in situ detection techniques applied immediately before decontamination to adequately estimate the level of contamination of the skin contaminant layer relevant for uptake. If the removal-resuspension / evaporation rate is low, but uptake rate is high, an interception sampler or an in situ- direct technique would give a good measure of dermal uptake. If the removal-resuspension/evaporation rate is high and uptake rate is low, an interception sampler (assumed to have a better retention performance compared to skin) would greatly overestimate uptake. In this case biological monitoring, being a non-route specific method for uptake, would be preferable, and also in the cases that both transport rates are high. Since the results obtained by different sampling principles are influenced by a range of mass transport processes and may have to be extrapolated beyond the sampled contact volume, all sampling methods are faced with fundamental problems, such as

- interception and retention characteristics of interception techniques differ from real skin or clothing;
- removal methods, e.g. tape stripping, solvent washing, and use of surfactants, may influence the characteristics of the skin; they may also be of limited use for repeated sampling;
- removal techniques, e.g. skin washing, are not appropriate for all body parts;
- extrapolation from small areas sampled, e.g. pads (patches) or skin strips, to the whole exposed area can introduce substantial errors;
- behaviour of a (fluorescent) tracer introduced in the mass transport when using in situ-techniques may differ from the behaviour of the substances of interest.

As indicated, the total mass measured may be a poor surrogate for the uptake, either since the mass of chemicals on the skin is not all available for uptake or is spread very unevenly on the skin. It would be more relevant to measure the exposure using a sampler that was a closer mimic of the skin, just as for inhalation exposure respirable dust sampling can be used to select the biologically relevant exposure to dust. Progress has been made in developing a prototype diffusive dermal sampler based on an adsorbent sandwiched between a semi-permeable barrier membrane and an impervious backing. Further development of this type of sampler may in the longer term offer a more appropriate measurement method.

Information about soil or sediment adherence, dermal transfer from surfaces, contact rates, and frequencies for important exposure scenarios is very limited. Only a few studies have been conducted to better characterise dermal contact and chemical transfer to the skin. These studies have focused on chemical release from sediments, sediment adherence to skin, and residue transfer from treated surfaces to skin. More studies are needed to better characterise activities associated with these environmental exposures.

Surface contact occurs when the skin comes into contact with a contaminated surface and chemical residue is transferred to the skin. This may contribute to oral exposure if chemical residues on the hands are transferred to the mouth or transferred from the hands to food. One major step to estimate dermal contact accurately is to better define the activity being performed at the time of exposure. If the activity patterns of humans were better characterised, the uncertainty of the dermal exposure characterisation process would be greatly reduced.

Dermal absorption

The physiology and biochemistry of the skin can account for much of the variability associated with dermal absorption of substances. The three routes of entry through the skin are the stratum corneum, the sweat duct, and the hair follicle.

The amount of chemical coverage on the skin surface can influence the amount of dermal absorption. Chemical coverage of the skin may be incomplete or exceed the exposed skin surface area by piling up on itself. Likewise the transfer efficiency from a contaminated surface to the skin or liquid solution may be highly variable due to the nature and extent of the contact or the deposition of chemical residue due to evaporation of the liquid. Quantitative exposure assessments for contaminants in water and air are based on the use of a permeability constant (K_p in cm/hr), which is a measure of the rate of penetration into the skin. K_p is usually measured in the laboratory from *in vitro* studies at steady state (infinite dose experiments). For exposure to soil, percutaneous absorption is usually expressed as the fraction of the applied dose absorbed from both *in vivo* and *in vitro* studies. For applications of soil containing equal concentrations of a contaminant, the amount of soil that adheres to the skin determines the amount of contaminant absorbed. Many of the permeability coefficients are based on predictive methods that commonly use octanol-water partition coefficients (P_{ow}) and molecular weight due to a lack of experimentally derived permeability coefficients for many chemicals. Most experimentally derived permeability coefficients are determined using the pure chemical deposited onto skin in a volatile solvent (e.g., acetone or ethanol) or the chemical in an aqueous solution. A number of factors may influence dermal absorption estimation such as physical and chemical characteristics of the contaminant (including factors such as corrosivity), matrix composition, physiological characteristics of the skin (including anatomical site or species), amount of surface area contact, and rate and mechanism of absorption.

Quantification of percutaneous penetration is an essential step in reducing the uncertainty of dermal risk assessment. Generally, if no quantitative absorption data are available for a substance it is assumed that 100% of the material applied to the skin surface is available systemically. This is an extremely conservative assumption, yet necessary due to the lack of data concerning absorption rates for chemicals.

Rates of permeation of chemicals cannot be precisely measured by analysis of absorbed material in excreta. Therefore, permeability constants are difficult to determine by those *in vivo* techniques, although *in vivo* Kp assessments can be improved by blood sampling, followed by pharmacokinetic analysis. *In vitro* techniques can be used to provide fast, direct measurements of flux and permeability constants (Kp) in human skin. In addition, factors affecting dermal absorption from various matrices (soil, water, oil etc.) can be controlled in *in vitro* studies. The most relevant percutaneous penetration data comes from human volunteer studies, but these data are rare. Costs and ethical constraints frequently rule out the testing of toxic compounds in humans. This necessitates the use of *in vivo* animal or *in vitro* methods which requires extrapolation of the results to those expected in humans.

Predicting dermal absorption with mathematical modelling

An approach for validating *in vitro* techniques is to use *in vitro* derived parameters of skin barrier function (i.e., the permeability coefficient and partition coefficient) in mathematical model representing *in vivo* absorption. In this approach, experimental variables in the *in vitro* and *in vivo* studies do not have to be identical as long as differences are described in the mathematical model.

Mathematical modelling can be used to describe the dermal absorption process by applying conservation of mass equations. Mathematical models that are mechanistically based require physicochemical parameters for the absorbing chemicals (e.g., diffusion coefficients and partition coefficients or parameters derived from these like the permeability coefficient). Depending on the situation that these mathematical models are describing, they will also include the volume of the vehicle, blood flow rates and so on. If the physicochemical parameters for a given compound are available, then these models can be used to describe dermal absorption for situations other than those used in the experiments in which the physicochemical properties (e.g., permeability coefficient and partition coefficient) were measured. For example, the mathematical model can use steady-state *in vitro* measurements to predict unsteady-state finite dose *in vivo* measurements, at least when lag time or equivalent information is known. These models are distinctive from QSAR models in that QSAR models are used to relate chemical structure to the physicochemical parameters that are important to dermal absorption, i.e., permeability coefficients and partition coefficients. For example, the permeability coefficient is a measure of a chemical's diffusivity and solubility in the skin layers relative to the vehicle. Diffusivity is known to vary with molecular size. Small molecules diffuse faster than big molecules. Solubility depends on how similar (or different) the chemical is to the skin layers (i.e., the stratum corneum and the viable epidermis) compared to the vehicle that the chemical is in when it is presented to the skin. QSAR models are used to estimate/predict the physicochemical properties needed in the dermal absorption model The mathematical model lets you use measurements made in one type of experiment to estimate dermal absorption in a different exposure scenario.

Predicting dermal absorption with QSAR techniques

Penetration of chemicals through the skin can be described as diffusion through a pseudo-homogenous membrane. This can be described using Fick’s first law that states, “the flux of the penetrating chemical at a location within the membrane barrier is proportional to the membrane diffusion coefficient and the concentration gradient at that position”. When skin is exposed to a chemical, chemical penetration through the stratum corneum will be initially

rapid and slow as it satisfies the capacity of the stratum corneum for the chemical. At this point absorption is unsteady and the chemical has not reached the systemic circulation. The chemical will then reach the systemic circulation and, if exposure continues, the concentration gradient through the skin will become constant. At this point absorption has reached steady state meaning the mass of chemical entering and leaving the skin are constant. A simplified mathematical model has been developed that successfully estimates dermal absorption from infinite dose aqueous solutions by taking into account both the non-steady state and steady state period of absorption. This model can be represented using 2 algebraic equations, one for the non-steady state absorption period and one for the steady state absorption period. In combination with QSAR models for the permeability coefficient and partition coefficient, this model can predict dermal absorption for chemicals that have not been studied. These simple equations have provided reasonable estimates of *in vitro* and *in vivo* data. It should be mentioned that nearly all of the QSAR equations for estimating permeability coefficients are restricted to aqueous solutions. This is because permeability coefficients are vehicle dependent and nearly all of the data are from aqueous vehicles.

The first type of equation/model is used to estimate the physicochemical parameters that characterise dermal absorption, i.e., permeability coefficients, partition coefficients, diffusion coefficients, lag times, and so on. The second type of equation/model uses these physicochemical parameters in an equation to estimate dermal absorption.

Most of the QSAR equations are the first type. They are structure-activity based equations designed to estimate chemical properties in skin. Many of the QSAR equations for skin estimate "steady-state" permeability coefficients from aqueous vehicles. However, there are QSAR equations for estimating partition coefficients between the stratum corneum (or sometimes skin) and aqueous vehicles and for estimating effective diffusion coefficients in the skin.

Dermal absorption is usually estimated using the second type of equation, which is some sort of mass balance model that uses parameter estimates, perhaps from a QSAR equation. These equations can and have been written to account for lag time and the material remaining in the skin as well as for concentrations changing in the vehicle applied to skin. For example:

*Cumulative mass absorbed = permeability coefficient * concentration * exposure time (Eq. 1)*

This equation comes from a steady-state mass balance. The permeability coefficient may be estimated using QSAR, but this does not make the above equation itself a QSAR equation. Eq. 1 does not account for lag time. If the exposure time is taken to be the time until the exposure ends, then Eq. 1 also does not account for material that will still be in the skin when the exposure ends. However, these flaws are not part of the QSAR equation, they are flaws of the mass balance equation.

Mass balance equations are not restricted to steady state and can be derived to include absorption of chemical in the skin at the end of the exposure. This is the case in the equations recommended for estimating dermal absorption from contaminated water. The recommended mass balance based equations (i.e., the second type of equation) use lag time (really an estimate of the diffusion coefficient) and permeability coefficient. Estimates of lag time and the permeability coefficient are calculated using QSAR equations. The permeability coefficient can be estimated with the same equation.

There are a very few equations of a third type, which estimate dermal absorption directly using only structure-activity parameters, but these equations can only be used for the situations in which they were derived.

The chief advantage of the strategy of estimating the physicochemical parameters by QSAR and then incorporating these into mass balance equations is that it can be used for a wide variety of exposure scenarios, provided we have the appropriate physicochemical data.

The chief problem right now is that permeability coefficients (or alternatively, partition coefficients) are not available for non-aqueous vehicles and we do not know how permeability coefficients are affected by dermal absorption of multiple compounds at the same time.

Mind set and goals of the workshop

The present workshop 2 will focus on the pertinent issues by bringing ideas and experimental data of researchers in these fields together in order to bridge the gap between exposure measurement and the assessment of dermal uptake of chemicals for risk assessment.

In keynote presentations by **Dr. Nick Warren** and by **Prof. Richard Guy**, the relevant issues for dermal exposure assessment and for dermal penetration will be presented with focus on the need for bridging.

The workshop itself will start with a series of short and focussed presentations covering the more detailed issues relevant for the bridging of the gap. These presentations will also cover the important variables that should be considered when assessing the relevance of dermal exposure data for penetration. For this goal, three well-known researchers have been asked to highlight some important issues in very short presentations which may then form the basis of discussions.
Dr. Derk Brouwer will cover temporal and spatial patterns of exposure versus dose.
Dr. John Cherrie will cover QSAR methodology – uses and limitations.
Prof. Annette Bunge will cover the characterisation of surface loadings/concentration – vehicle, distribution, skin condition, and measurement approaches.

All speakers will prepare in advance, at least two statements which will stimulate and focus the discussions on how to integrate external exposure and absorption data in an optimal way.

On the basis of the results of the workshop, a *posterior white paper* will be developed for further discussions and research.